Reports

Possible Virus-Induced Genetic Abnormalities in Tree Fruits

Abstract. Seedlings of *Prunus avium* and *P. cerasus*, grown from seed of infected trees, showed symptoms of cherry necrotic rusty mottle and sour cherry yellows diseases. No virus could be detected in the *P. avium* seedlings showing virus-like symptoms and only 10 percent of the 30 percent of *P. cerasus* seedlings showing symptoms contained virus. These results suggest that viruses may induce certain genetic abnormalities.

Necrotic rusty mottle is a virus disease of cherry (*1*) which, in several varieties, induces distinct and easily recognized symptoms. The cherry variety used most commonly as a standard index host for NRMV (necrotic rusty mottle virus) is Lambert (*Prunus avium*). In Lambert the virus produces medium-to-large angular necrotic lesions that may coalesce and involve large areas of the leaf. Infected trees may develop discrete necrotic lesions in the bark, in the form of superficial gum-filled blisters or deeper gum pockets, or a general bark necrosis. Under favorable conditions Lambert invariably develops both leaf and bark symptoms. The variety Bing, usually affected less severely, also develops characteristic leaf and bark symptoms. The disease is essentially latent in some varieties of sweet cherry, such as Black Tartarian. This paper reports virus-like symptoms in the absence of demonstrable virus in seedlings from virus-infected sweet and sour cherry trees.

A population of sweet cherry seedlings at the University of California, Davis, exhibited symptoms of NRMV, but in many of the seedlings the virus itself could not be demonstrated by standard graft transmission tests. At least three bud scions were used in each Lambert tree, and one or more bark shields lived for at least 1 year. In other cases Lambert and Bing scions were grafted on seedling trees showing symptoms of NRMV. The growth from the scions was observed for at least 4 years.

In 1952 a controlled cross was made of Bing × Black Tartarian and its reciprocal. The Black Tartarian parent tree was known to be infected with NRMV. So far as known, the Bing parent tree was infected only with PRSV (Prunus ringspot virus). Symptoms resembling those of NRMV showed in 26 of 214 seedlings from the Black Tartarian seed, and in 25 of 188 seedlings from the Bing seed. The symptoms were essentially the same as those produced by NRMV in Bing and Lambert, but all attempts to transmit virus from seedlings with symptoms failed. The virus was not transmitted to the seedlings, at least not in a normal form, since no graft transmission could be made from the affected seedlings. The virus-like symptoms were perpetuated when the seedlings were propagated vegetatively. Seedlings from healthy parent trees have not shown NRMV-like symptoms in several controlled crosses made during the past 10 years at Davis (*2*).

Results were somewhat similar with Stockton Morello Cherry (*Prunus cerasus*) seedlings produced from seed of trees with multiple infections of PRSV, GRMV (green ring mottle virus), and SCYV (sour cherry yellows virus). About 25 percent of 700 seedlings grown in 1950 from 1949 seed showed symptoms, some resembling GRM disease and other SCY disease. Additional seedlings of this same population developed symptoms in 1951, as did seedlings that showed symptoms the previous year. No seedlings were available from healthy trees for comparison, because no healthy sources of Stockton Morello were known at that time.

Indexing tests were made in 1951 to attempt to confirm the presence in the seedlings of the three viruses known to be present in the parent trees of Stockton Morello. Thirty-five seedlings, some with GRM disease symptoms and some with SCY disease symptoms, were transplanted to a field plot for tests. All were indexed on Shiro-fugen flowering cherry (*3*) to determine the presence of PRSV.

Eleven trees indexed positive; the others were negative. Montmorency sour cherry (*Prunus cerasus*) was used to index for GRMV and SCYV. Only 19 healthy Montmorency index trees were available at that time. These were bud-grafted with buds from eight seedling trees that tested positive for PRSV and 11 seedling trees that indexed negative for PRSV. Controls were uninoculated trees from another experiment. Symptoms of SCYV developed in two of the eight trees inoculated with ringspot-infected seedlings, and no symptoms developed in the 11 trees budded with tissue of the ringspot-free seedlings. In subsequent years the remaining 16 seedling trees of the original 35 were indexed for GRMV and SCYV, and no transmission was obtained. It seems that the virus-like symptoms in Stockton Morello seedlings in which no virus was detected must be considered genetic abnormalities. These virus-like symptoms, often indistinguishable from symptoms produced by the virus, were perpetuated when the seedlings were scion-propagated and have persisted unchanged for 11 years in branches arising from the scions.

There are in stone fruits several other interesting examples of virus diseases that appear to have nonvirus counterparts nontransmissible by grafting. Among these are peach willow twig, almond bud failure, almond calico in cherry, and some types of line pattern virus in cherry, swollen node and rough bark of Burbank cherry, peach calico, and prune dwarf. Studies of the viruses causing these diseases have been insufficient to establish whether the nontransmissible counterparts can be traced back to virus-infected parent trees or progeny from infected trees.

Carpenter and Furr (*4*) recently reported that seedlings of Palestine sweet

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Type manuscripts double-spaced and submit one ribbon copy and two carbon copies.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each. Submit three copies of illustrative material.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

lime exhibited nontransmissible wood pitting. Some seedlings were from an old tree that was severely pitted. Whether the parent tree was infected with xyloporosis virus, which produces wood pitting symptoms, was not stated. Virus and nonvirus variegation is common in camellia, abutilon, and some other ornamental plants.

At this time one can only speculate about the mechanism involved in the production of seedlings with the genetic abnormalities described here. One possibility might be an effect of virus nucleic acid on host nuclear material, with the virus nucleic acid actually becoming a part of the host genome and so coming under the control of the host nucleus.

The condition described here in cherry may be somewhat analogous to lysogeny in certain bacteria, though information is too meager for a really meaningful comparison. In *Corynebacterium diphtheriae*, for example, Freeman (*5*) and Groman (*6*) showed a correlation between toxin production and lysogeny. In that case the prophage profoundly altered the physiology of the cell, yet the phage was in a noninfectious state. Likewise with the condition in cherry: the virus-like symptoms are evident and abundant though no infectious agent can be demonstrated by grafting. The symptoms are perpetuated by bud propagation and also pass through the seeds. If there is some interaction of virus and host genome, this interaction may, without preventing symptom expression, limit movement of the virus in the host to its transfer from cell to cell during mitosis. Such a situation would prevent transmission of the virus across a graft union.

If viruses can effect permanent genetic changes in the higher plants, their role in the evolutionary process must be considered.

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References and Notes

1. B. L. Richards and E. L. Reeves, in "Virus diseases and other disorders with virus-like symptoms of stone fruits in North America," *U.S. Dept. Agr. Handbook No. 10*, 120 (1951).
2. These crosses were made by Dr. R. M. Brooks, Department of Pomology, University of California, Davis.
3. J. A. Milbrath and S. M. Zeller, *Science* **101**, 114 (1945).
4. J. B. Carpenter and J. R. Furr, *Plant Disease Reptr.* **44**, 916 (1960).
5. V. J. Freeman, *J. Bacteriol.* **61**, 675 (1951).
6. N. B. Groman, *ibid.* **66**, 184 (1953).

13 July 1962

Determination of the Mixing Ratios of Water Vapor and Carbon Dioxide in the Stratosphere

Abstract. Values for water vapor mixing ratio (0.32 g/kg) and carbon dioxide content (0.030 percent by volume) were measured at 119,300 feet and are compared with values obtained by others at lower altitudes.

Many investigators have measured the water vapor content of the stratosphere (*1–3*). However, no measurements have been reported for altitudes above 100,000 feet. Most investigators have used hygrometers of the frost-point type. On 2 May 1962 our group collected a sample of water from the air at an altitude of nearly 120,000 feet by a balloon-borne sampling system and recovered it for analysis (*4*).

An adsorbent pump (see Fig. 1) with an adsorbent bed of synthetic zeolite (*5*) cooled by liquid nitrogen was developed to sample a large volume of stratospheric air. The pump's capacity is 130 standard cubic feet of air at altitudes up to 150,000 feet. A second, specially prepared adsorbent bed was used to collect water vapor and carbon dioxide from the sampled air. This second bed, 2 inches thick, was sealed by two ball valves, one each at the inlet and outlet of the air stream. The pump's adsorbent bed (the first bed) was designed to keep the adsorbent at liquid nitrogen temperature throughout the sampling period. The collection bed was at ambient temperature. A pressure relief valve on the liquid nitrogen chamber kept the nitrogen from either building up to high pressures or solidifying at the reduced pressures of high altitudes.

To eliminate balloon-borne contamination, the adsorbent pump was located 150 feet below the balloon. After the balloon system had reached a float altitude of 119,300 feet, the two valves on the collection bed were opened. The inlet valve allowed ambient air to enter the collection bed chamber. The outlet valve, which is the inlet to the pump proper, allowed the air in the collection chamber to pass into the pump, where it was adsorbed. The adsorption process pulled ambient air through the collection bed where the water vapor and carbon dioxide were removed. The dry, CO_2-free air was then absorbed in the pump's zeolite bed.

At the end of a 180-minute sampling period, the valves were closed, and the adsorbent pump system was recovered by parachute. A flowmeter was used to determine flow rate and total volume flow during the sampling period. In addition, all of the sampled air (127 standard cubic feet) retained by the adsorbent pump was subsequently measured. Values from both recovered air and flowmeter measurements were in good agreement.

Both the water vapor and carbon dioxide were recovered from the collection bed by regenerating the adsorbent of the collection bed. The regeneration process consisted of heating and pumping on the adsorbent and collecting the desorbed water vapor and carbon dioxide in a liquid-nitrogen cold trap. The water vapor and carbon dioxide were then separated by differential distillation and measured.

The calculated mixing ratio of carbon dioxide on the 2 May flight was 0.030 percent by volume. This is within experimental error, considering flow measurement and altitude determination errors, of the generally accepted value of 0.031 percent.

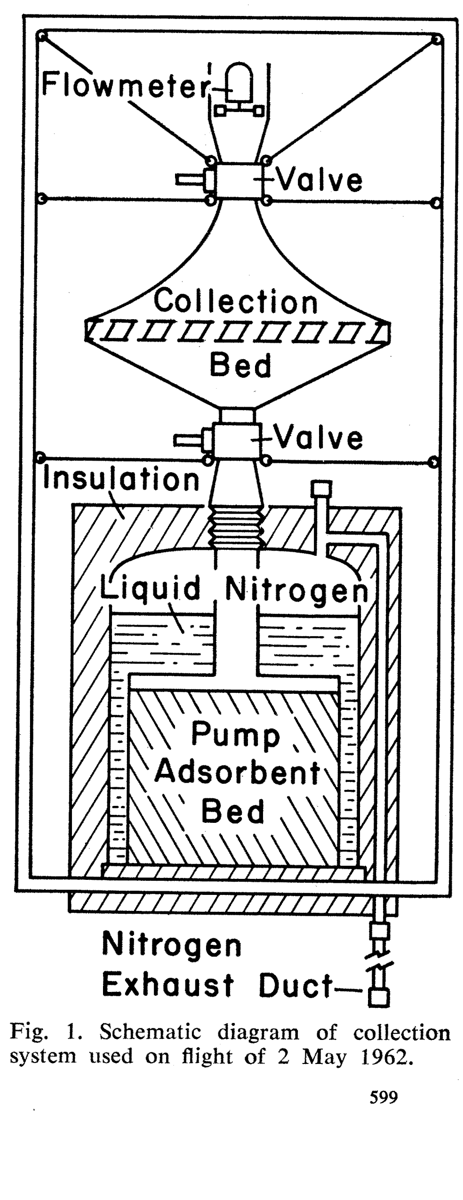

Fig. 1. Schematic diagram of collection system used on flight of 2 May 1962.